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SO Poultry Science, (1997) Vol. 76, No. 5, pp. 677-682.

SO VETERINARY RECORD, (16 JAN 1993) Vol. 132, No. 3, pp. 56-59.

SO Journal of Parasitology, (1992) Vol. 78, No. 5, pp. 906-909.

SO AVIAN PATHOL, (1986) 15 (2), 271-278.

SO ACTA PARASITOL POL, (1976 (RECD 1977)) 24 (11-19), 103-117.

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ACTA PARASITOLOGICA POLONICA

ACTA PARASITOLOGICA POLONICA
Vol. XXIV, fasc. 11 Warszawa, 31. XII. 1976 pp. 103–117

Janina PASTUSZKO

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Caecal coccidiosis in domestic fowl *Gallus gallus* (L.) caused by *Eimeria tenella* (Railliet et Lucet, 1891) III. Attempts to induce immunity in chicks by the use of X-ray attenuated oocysts

Kokcydioza jelit ślepych wywoływana u kura domowego *Gallus gallus* (L.) przez *Eimeria tenella* (Railliet et Lucet, 1891). III. Próby uodporniania kurcząt przy pomocy oocyst inaktywowanych promieniami Roentgena

Abstract

Pastuszko J. 1976. Caecal coccidiosis in domestic fowl *Gallus gallus* (L.) caused by *Eimeria tenella* (Railliet et Lucet, 1891). III. Attempts to induce immunity in chicks by the use of X-ray attenuated oocysts. *Acta parasit. pol.*, 24, 103–117.

In controlled experiments, enteral inoculation of 14-day-old chicks with a vaccine at the standard dose of 100,000 oocysts irradiated with 10,000 to 30,000 R, using both single and double immunization, or at the single dose of 75,000 oocysts irradiated with 20,000 to 35,000 R, substantially protected the birds against subsequent challenge with high doses of fully infective oocysts of the same coccidian species. The course of coccidiosis in these chicks was light of abortive type, its normal symptoms decreasing with increased level of irradiation of oocysts used for immunization. The endogenous developmental cycle of parasites was significantly inhibited, the oocysts burden in droppings being much lower than in controls. The non-immunized control chicks exposed to infection with fully infective oocysts showed acute and severe course of coccidiosis, the mortality rates being about 80–86%. The mean body-weight gains in the immunized chicks was higher than that in the non-immunized controls.

In recent years many studies have appeared in literature, dealing with the pathology and immunological phenomena in the host organism in the course of coccidiosis (Leathem and Burns 1968, Long 1968, 1970, Hein 1968, Reid and Johnson 1970, Rose 1967, 1971, Rose and Long 1970, Euzéby et al. 1967, Klimeš and Orel 1969, and many others). According to the opinion of many authors, X-rays

appear to be particularly useful in practice in the attenuation of oocysts. So it was decided to study this problem in connection with the search for the best ways and means of obtaining oocysts with lowered infectivity which could be used for the immunization of chicks against caecal coccidiosis produced by *Eimeria tenella*. This subject of study seems well motivated in view of the economic importance of the problem of coccidiosis of poultry (P a s t u s z k o 1973 a, b), and also because *E. tenella* is that species of coccidia whose biology has been most comprehensively explored. This makes it possible to compare the results of the present study with those published by other authors.

Material and methods

Oocysts

Eimeria tenella oocysts obtained directly from the caeca of 24-day-old Leghorn chicks suffering from caecal coccidiosis following laboratory infection in the second week of their life, were kept at 24–26° C in Petri dishes in a 2% potassium dichromate solution up to the completion of sporulation. To guarantee sufficient supply of oxygen to the oocysts, the solution whose surface level did not exceed one third of the height of the dish, was stirred two or three times daily. After the completing of sporulation, before exposure of oocysts to X-rays and their later use for infection of experimental chicks, the sediment from the bottom of the Petri dish was several times rinsed with distilled water. Then the oocysts were attenuated by the use of X-rays. A Roentgen apparatus emitting up to 1,000 R/min was used to this end. Exposure filtered through a copper plate 0.1 mm thick was applied, and the doses were 10, 15, 20, 25, 30 and 35 thousand Roentgens.

Experimental birds and infection

The experimental Leghorn chicks were kept from the very moment of hatching in conditions excluding accidental contact with coccidia. From the second day after hatching the chicks were fed with "DK" concentrated fodder. The birds used in the experiments were two weeks old. The experiments were carried out on 162 chicks, divided into seven experimental groups and two controls. The oocysts were introduced to the crop. The effects of immunization and infection of the chicks were tested on the ground of clinical records and anatomopathological and histopathological findings at post-mortem examination.

Experimental procedures:

The experiment was carried out in two series:

Series I: The experiments carried out in the spring and summer (April-June 1968) provided an opportunity for the observation of chicks immunized with 100,000 *E. tenella* oocysts per bird. Three batches of oocysts, prior to being given to the chicks, were exposed to different doses of X-rays.

The whole group of 70 14-days-old chicks, used in this series of the experiment, was divided into three groups, 16 chickens each, the remaining 22 serving as controls. The birds in the respective experimental groups were immunized with a single or with two successive doses of oocysts attenuated by X-rays, and later were given fully infective oocysts of *E. tenella* (100,000 oocysts per chick). Chicks in Group 1 were immunized with oocysts attenuated by exposure to 10,000 R, those of Group 2 to 20,000 R, and of Group 3 to 30,000 R. The controls were not immunized, but obtained the same dose of fully infective oocysts.

The programme of the experiment pertaining to each group is seen in Table I.

Series II: The experiments carried out in the autumn (September–October 1968) included observations of chicks immunized with a dose of 75,000 *E. tenella* oocysts per chick. Four batches of oocysts, before they were given to the chicks, had been exposed to different doses of X-rays.

Experimental day	
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Table I

Design of the Series I experiments

Experimental day	Chicks of experimental groups 1, 2, 3 (each group of 16 chicks)		Chicks of a control group (22 birds)
	subgroup A (8 birds)	subgroup B (8 birds)	
1	exposed to *E. tenella* oocysts attenuated with X-rays		exposed to fully infective *E. tenella* oocysts
16	exposed to *E. tenella* oocysts attenuated with X-rays (doses of oocysts and X-rays: as those applied on day 1)	no immunization	exposed to fully infective *E. tenella* oocysts
29	exposed to fully infective *E. tenella* oocysts	exposed to fully infective *E. tenella* oocysts	—
40	closure of observations		

For explanation: dose of oocysts - 100,000/chick for all groups, including control; doses of X-rays: ist group - 10,000 R, 2nd group - 20,000 R, 3rd group - 30,000 R.

The whole group of 92 birds composed of 14-day-old chicks used in this series was divided into four experimental groups, 18 chicks each, and the remaining 20 chicks served as controls. The chicks of the respective experimental groups were immunized with a single dose of attenuated oocysts. On day 20 after immunization, they were infected with fully infective oocysts of *E. tenella*; some obtained 140,000 oocysts per chick, to the crop (Subgroup A), others (Subgroup B) were naturally infected through direct contact with specially kept chicks suffering from coccidiosis of caeca and put in the cages together with the immunized birds. Chickens ranked to Group 1 were immunized by oocysts exposed to 15,000 R, those of Group 2 to 20,000 R, of Group 3 to 25,000 and of Group 4 to 35,000 R.

The design of the experiments of Series II is given in Table II.

Table II

Design of the Series II experiments

Experimental day	Chicks of experimental groups 1, 2, 3, 4 (each group of 18 chicks)		Chicks of a control group (20 birds)
1	exposed to *E. tenella* oocysts attenuated with X-rays		exposed to fully infective *E. tenella* oocysts
21	exposed to fully infective *E. tenella* oocysts		exposed to fully infective *E. tenella* oocysts
	subgroup A: 140,000 oocysts per bird inoculated in the crop	subgroup B: birds contacted with chicks showing caecal coccidiosis	
33	final post-mortem examination		

For explanation: immunizing dose of attenuated oocysts - 75,000/chick, doses of X-rays: 1st group - 15,000 R, 2nd group - 20,000 R, 3rd group - 25,000 R, 4th group - 35,000 R.

Means of graphic presentation of the results

A. Quantitative results (testing the number of oocysts per g of droppings): Assuming that the horizontal axis of abscissas (x) of a rectangular system on a plane represents the time interval, and the vertical axis of ordinates (y) represents the values obtained in the test, the experimental points have been determined. These points connected by a straight line segments form a broken line which shows the changes of the examined parameter. This broken line is seen in a diagram as a thin line.

Next an approximating curve has been drawn on the basis of the following correlation:

$$\sum_{i=1}^{k} \sqrt{(x'_i - xd_i)^2 + (y'_i - yd_i)^2} = \min$$

where: x'_i, y'_i = co-ordinates of the approximating curve in point; xd'_i, yd'_i = co-ordinates of experimental points, k = number of experimental points.

The above correlation requires that the sum of the distances of the points situated on the curve from experimental points be reduced to the minimum. The approximating curve has been presented in a diagram by a thick line.

B. Qualitative results: have been arranged according to the following gradation:

—	0
+	1
++	2
+++	3
++++	4

After determining the above gradation the results were treated as numerical and the course of the changes of any parameters was drawn by linking the successive experimental points by segments of a straight line. The position of the experimental points was determined in the rectangular system of co-ordinates, the time intervals being shown on the axis of abscissas, and the above cited gradation on the axis of ordinates.

Results

The results of observations made in Series I of the experiments are seen in Diagrams 1–4, showing that chicks enterally immunized with oocysts attenuated by exposure to X-rays at doses ranging from 10,000 to 30,000 R, proved to be highly resistant to the successive infection with fully infective oocysts of that parasite. This was manifested by a light and even abortive course of coccidiosis and a substantial reduction of the number of oocysts disseminated by infected individuals in their environment. On the contrary, the controls (C) infected twice with fully infective *E. tenella oocysts*, the individual dose being 100,000 per chick, revealed a typical course of the disease with quickly advancing emaciation and high mortality rate (86.4% during 40 days of observation).

In chicks of Group I immunized once or twice with oocysts exposed to a dose of 10,000 R, and later infected with fully infective *E. tenella* oocysts, only suffered from light coccidiosis; the birds which were twice immunized, did not eliminate oocysts with droppings beginning with the tenth day after the second immunization. The double immunization

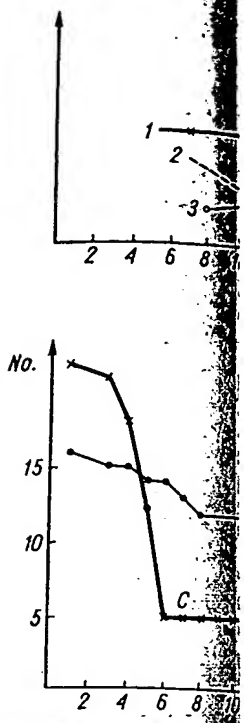

Diagram 1. Showing
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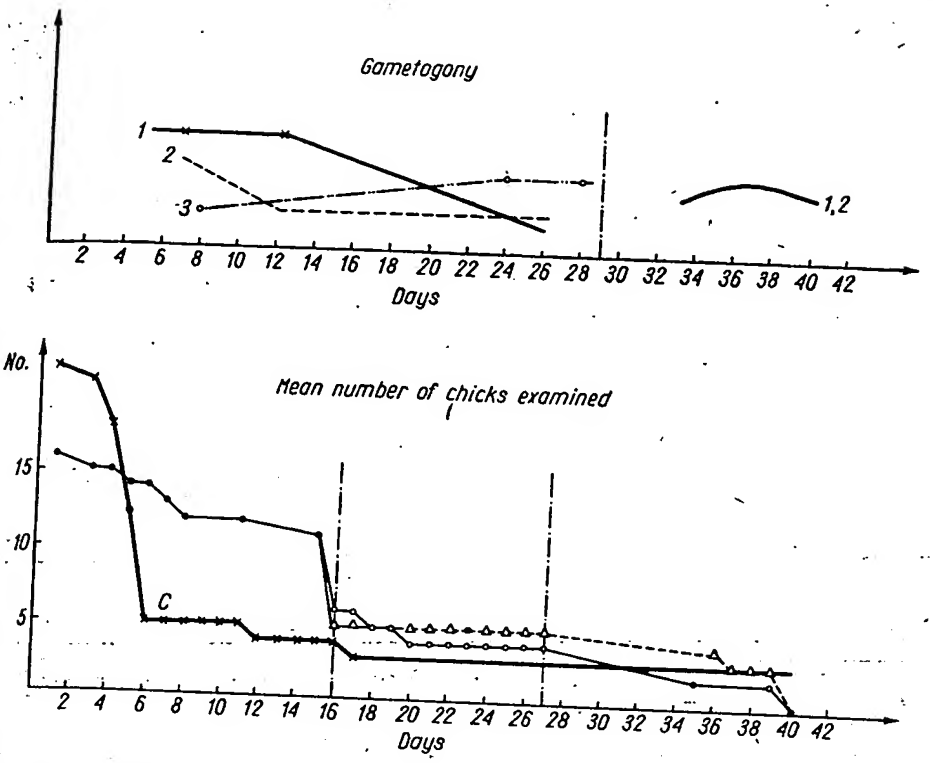


Diagram 1. Showing the course of *E. tenella* gametogony in chicks of Series I (Groups 1–3), the mean number of examined birds being considered. The means of graphic presentation of the results are explained in Chapter dealing with "Material and methods". C — control group.

evidently inhibited schizogony and gametogony of coccidia (Diagrams 1, 2 and 3).

Similar results were obtained in the experiments with chicks belonging to Groups 2 and 3, which were immunized with *E. tenella* oocysts exposed to 20,000 R and 30,000 R. It should be noticed that the morbid symptoms observed within the period between the first and second immunization in the chicks of Groups 2 and 3 were considerably lighter than Group I. No deaths occurred (the death of a single chicken on the 14th experimental day was the result of accidental injuries).

The results obtained in Series II of the experiment (Diagrams 5–8) are in principle similar to those obtained in Series I. In the group of controls, an acute course of coccidiosis was recorded, and the death rate during 33 days of observation amounted to 80%. On the other hand, in the experimental groups, with the exception of Group 1, comprising chicks immunized with oocysts exposed to 15,000 R, no evident clinical symptoms of coccidiosis were recorded, apart from the elimination of *E. tenella* oocysts in droppings up to the 20th day after immunization. In this connection it should be concluded that a single dose of 75,000 *E. tenella* oocysts attenuated with X-rays in a dose up to 15,000 R does

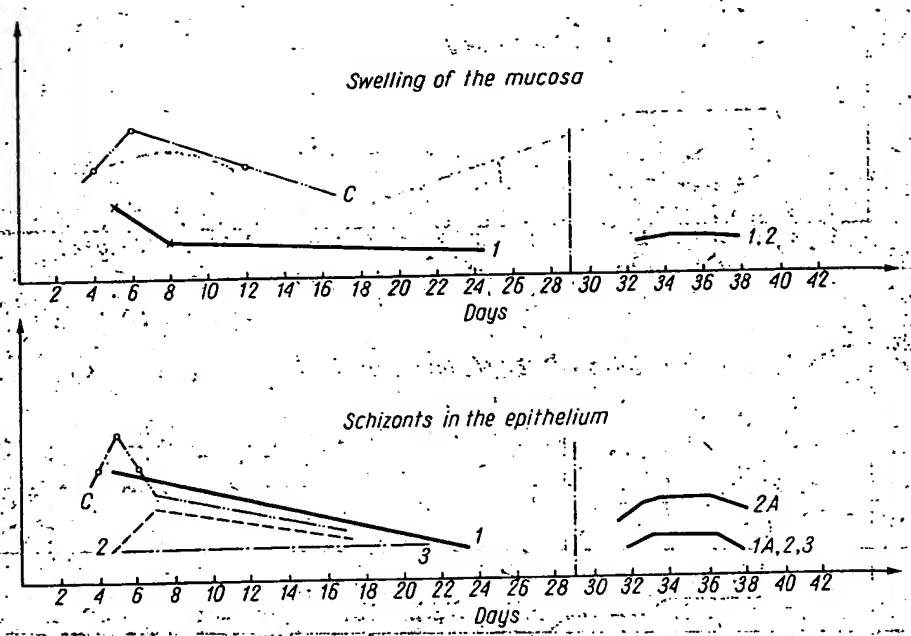
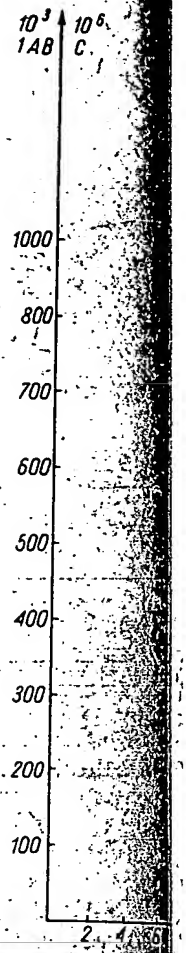


Diagram 2. Showing changes in the mucosa and the course of *E. tenella* schizogony in chicks of Series I (Groups 1–3). The means of graphic presentation of the results are explained in Chapter dealing with "Material and methods". C — control group.

not reveal useful immunizing properties producing resistance to coccidiosis.

The inoculation of chicks with a vaccine containing oocysts irradiated with 20,000 R evidently inhibited the course of endogenous developmental cycle of the parasite and considerably reduced the elimination of oocysts with droppings after the challenge infection, as it was recorded during the course of the experiment. The resistance to the challenge infection was even more evident when chicks were given oocysts attenuated with 25,000 R and 35,000 R. The results of autopsies of the chicks which died as a result of infection and those killed in the course of the experiment, confirmed the role of cellular elements, particularly lymphoid cells in the mechanism of the formation of resistance to coccidiosis, as recorded earlier by E u z é b y et al. 1967 a, b. This pertains to the experiments of Series I and Series II. Apart from the generally known pathological changes in the caeca of immunized chicks and of those infected with fully infective oocysts, the histological examination of the intestine walls revealed an inflammatory infiltration containing cell elements and a general lymphoid hypertrophy.

The results of the experiments, as presented in diagrams, reveal the abortive course of coccidiosis in immunized chicks. An additional picture of the effectiveness of the immunization is provided by the body-weight gains of the experimental birds, whose mean values are given in Table III.



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Volume 76, Number 8

COCCIDIOSIS IN SWINE:

effect of disinfectants on *in vitro* sporulation of *Isospora suis* oocysts

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Coccidiosis, a disease caused by *Isospora suis* infection, is being diagnosed with increased frequency in piglets.[1] At 5 to 10 days of age, piglets with coccidiosis develop yellow-tan scours that are unresponsive to most antibacterials.[2] Piglets become dehydrated and either lose weight or fail to gain. Morbidity is variable. Mortality is usually low but may reach 20% or greater in young animals.

Prevention is more important than treatment in control of coccidiosis. Control of enteric disease in piglets requires strict sanitation and restricted access to farrowing facilities. Anticoccidial drugs such as Amprol® (Merck) and Deccox® (Rhone-Poulenc) have been used experimentally with variable success. The study reported here was designed to determine the effect of commonly used disinfectants on the sporulation of *I. suis* oocysts.

Materials and methods

Approximately 500,000 oocysts of *I. suis* in 1 to 2 g of fresh feces from clinically infected piglets were placed in Petri dishes containing 130 ml of test solution. Cultures were kept at room temperature (21 C). Test solutions used were:

- DC and R spray fumigant concentrate (Rhone-Poulenc, Inc., Hess and Clark Division, Ashland, Ohio)
- IOFEC-80® (Whitmoyer Laboratories, Inc., Myerstown, Pa.)
- One Stroke Environ® (Vestal Laboratories, Division of Chemed Corporation, St. Louis, Mo.)
- Nolvasan® Solution (Fort Dodge)
- Ammonium hydroxide (Parsons' clear detergent ammonia, Armour-Dial, Inc., Phoenix, Ariz.)
- 5.25% sodium hypochlorite (The Chlorox® Company, Oakland, Calif.)
- Lysol (Lenn and Fink Products, Division of Sterling Drug, Inc., Montvale, N.J.)

Potassium dichromate (2.5%) and sulfuric acid (2.0%) were used as positive-control sporulation media.[3] Other solutions were used according to the manufacturer's recommendation or at other empirical dilutions. Each mixture was stirred daily. At 60 hours and again at 120 hours, 100 oocysts were counted to determine the percentage of sporulated oocysts.

Results

Sporulation of oocysts occurred in many disinfectants diluted according to manufacturers' recommendations (*Tables 1 & 2*). Oocysts did not sporulate in household ammonia at full strength or at 50% dilution. Little sporulation was seen when 8 oz ammonia/gal water was used, and oocysts that did sporulate appeared nonviable. The sporonts were often vacuolated or the granules were dispersed. The

oocyst wall was collapsed and folded. At the lowest concentration used (2 oz ammonia/gal water) oocysts appeared normal; 50% sporulated after 60 hours.

Lysol disinfectant used at a rate of 4 oz/gal water permitted some sporulation. Many oocysts were in the sporocyst (2-cell) stage. Sporonts were often granular or vacuolated.

Sodium hypochlorite (Chlorox) at full strength apparently caused lysis of the oocysts because none could be identified after repeated attempts to concentrate them. A 50% dilution of Chlorox was associated with poor sporulation and fragmented sporocyst walls. Although 63 to 68% sporulated at concentrations of 8 oz Chlorox/gal water, oocysts were often collapsed. Also, the sporozoites were indistinct and vacuolar and appeared free within the oocyst rather than within sporocysts. At a concentration of 1 oz Chlorox/gal water, oocyst sporulation was not inhibited and oocysts were morphologically normal.

Full-strength Environ inhibited sporulation of oocysts. Some oocysts were in the sporocyst stage but appeared degenerate. Sporulation was not inhibited when Environ was used at the recommended dilution, though refractile bodies were increased within some sporocysts, suggesting some degeneration.

In DC and R spray fumigant concentrate at full strength, about 50% of the oocysts had sporulated at 60 hours. Many oocysts or sporocysts were collapsed or fragmented. DC and R at the recommended dilution failed to inhibit sporulation.

IOFEC-80 at the recommended level and at half-strength failed to inhibit sporulation of oocysts. At half-strength, oocyst walls often appeared fragmented, and sporocysts absorbed the iodine stain. Nolvasan undiluted and at half-strength also failed to inhibit sporulation. Both IOFEC-80 and Nolvasan cleaned and cleared oocyst preparations of debris.

TABLE 1—Results of Sporulation of *Isospora suis* Oocysts in Household Disinfectants

	Sporulated (%)	
	After 60 hours	After 120 hours
Dichromate 2.5%*	86	90
Sulfuric Acid 2%*	82	84
Household Ammonia 100%	0	0
Household Ammonia 50%	0	0
Household Ammonia 8 oz/gal water	0	5
Household Ammonia 2 oz/gal water	55	77
Lysol 4 oz/gal water	8	12
Chlorox 100%	0	0
Chlorox 50%	15	0
Chlorox 8 oz/gal water	63	68
Chlorox 1 oz/gal water	86	88

*Served as control solutions for sporulation.

TABLE 2—Results of Sporulation of *Isospora suis* Oocysts in Commercial Disinfectants

	Sporulation (%)	
	After 60 hours	After 120 hours
Dichromate 2.5%*	86	90
Sulfuric Acid 2%*	82	84
Environ 100%	3	0
Environ (0.5 oz/gal water)**	82	77
DCR 100%	54	20
DCR (1 oz/gal water)**	87	84
IOFEC-80 50%	85	63
IOFEC-80 (1 oz/5 gal water)**	86	89
Nolvasan 100%	93	90
Nolvasan 50%	81	84

*Served as control solutions for sporulation.
**Manufacturer's recommended dilution.

Discussion

Concentrated household ammonia and Lysol best inhibited sporulation of *I. suis* oocysts. Sporulation was not inhibited by several commonly used commercial disinfectants when diluted per the manufacturers' instructions. Greater concentrations of these products were needed to inhibit sporulation or produce morphologic abnormalities.

Infectivity of oocysts in susceptible piglets after sporulation in these disinfectants needs to be evaluated. To control coccidiosis in piglets, swine producers should use highly concentrated disinfectant after cleaning farrowing facilities with steam or water under high pressure. Many producers are also using direct heat to destroy residual oocysts.

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